REMARKS UNDER 37 CFR § 1.111

Formal Matters

Claims 25-44 are pending after entry of the amendments set forth herein.

Claims 32-33 and 34-40 are amended. Claims 32-33 are amended to depend from claim 30. Support for the amendments to claims 34-40 is found in the specification at, for example, page 10, lines 1-9.

New claims 41-44 are added. Support for these new claims is found in the specification at, for example, page 27, top of Table 3, and claim 27 as previously presented.

No new matter has been added.

Applicants respectfully request reconsideration of the application in view of the amendments and remarks made herein.

Information Disclosure Statements

Applicants respectfully request that the Examiner initial and return the PTO SB-08A form submitted with the Information Disclosure Statement filed on may 20, 2003 in this application, thereby indicating that the references cited therein have been reviewed and made of record.

Applicants also request consideration of the references cited in an Electronic Information Disclosure Statement, filed on August 7, 2003 using the Office's EPAVE software. Please review the references cited therein, and forward a copy of the initialed form to applicants with the next action.

Specification

The Examiner requested clarification of the relationship between the instant application and the parent application U.S. application serial no. 09/217,037. The Examiner noted that while the first line of the specification states the present application is a continuation-in-part, the Bibliographic Data Sheet indicates the application is a CON.

A Request for a Corrected Filing Receipt was filed on April 5, 2001, and a Corrected Filing Receipt mailed on August 13, 2001. The Corrected Filing Receipt indicates the present application is a continuation-in-part of U.S. application serial no. 09/217,037.

In order to ensure the appropriate relationship is of record, applicants have enclosed an Application Data Sheet (ADS) for filing in the instant application, which ADS indicates that the present application is a continuation-in-part of the parent U.S. application serial no. 09/217,037.

Rejections Under §112, ¶1 – Enablement

Claims 25-40 were rejected on the grounds that the claims contain subject matter which was not described in the specification in such a way as to enable one of skill in the art to make and/or use the invention. This rejection is respectfully traversed.

The Office Action asserts that applicants have only provided guidance of how to produce a small number of conjugates within the scope of the claims, provided no guidance or working examples as to how to use such conjugates, and have not provided any *in vivo* or *in vitro* data demonstrating these conjugates are effectively taken up by neuronal cells or that the conjugates are cleaved in the cells in order for the steroids and/or neurotrophin to be effective at their intended site of action.

Applicants respectfully disagree. First, applicants note that the pending claims are not directed to any and all neurotrophin-steroid conjugates, but rather are directed to conjugates of a neurotrophin or neurotrophin receptor binding-fragment and a 4-pregnen-21-hydroxy or a 1,4-pregnadiene-21-hydroxy steroid. Furthermore, the claims require that the conjugant group pends from the steroid 21 hydroxyl group.

The specification provides numerous examples of the neurotrophin component of the conjugates (see, e.g., specification page 16, line 14 to page 17, line 39) as well as a detailed description of the knowledge in the art regarding the structure of neurotrophins which provides guidance for fragments (see, e.g., specification page 17, line 40 to page 18, line 29). The specification also provides guidance as to the position for conjugation within the amino acid sequence of such neurotrophins and fragments.

4-pregnen-21-hydroxy or a 1,4-pregnadiene-21-hydroxy steroids within the scope of the claims are described in the specification, with guidance as to the 21 hydroxyl group from which

the conjugant group pends, as recited in the present claims, including linkers that can be used to accomplish this conjugation (see, e.g., specification page 26, lines 9-16, and Tables 3 and 4).

Finally, the specification described methods for using the claims compounds (see, e.g., specification page 35, lines 1-23). Applicants further note that the ordinarily skilled artisan would recognize that the steroid conjugates of the claim could be administered in a manner consistent with methods of administration known for steroids and for neurotrophins.

Applicants respectfully request that the Examiner in particular consider dependent claims in the context of this rejection (e.g., claims that recite particular steroids (e.g., claims 30-33), particular neurotrophins (e.g., claims 34-38), and the like). Applicants note that such claims are of a substantially different scope than, for example, claim 25.

In view of the above, the Examiner is respectfully requested to withdraw this rejection.

Rejections Under §112, ¶2

Claims 34-40 were rejected as being indefinite, particularly for recitation of abbreviations and acronyms of neurotrophins and receptors in the claims. The Examiner kindly noted that the terms were discussed at least on page 10, line 6.

Claims 34-40 are amended to provide the full name for the elements previously only identified by acronyms. As noted by the Examiner, the specification discusses that these various factors were known in the art at the time of filing. An exemplary review by Shieh et al. (1997 Curr. Biol. 7:R627-630, copy attached), cited in the specification, evidences that NGF is nerve growth factor, BDNF is brain-derived neurotrophic factor, and NT refers to neurotrophin, with the "-3", "-4", and "-6" referring to particular types of neurotrophins (see page 1, col. 1). Further evidence is provided by a review by Leβmann et al. (Aug. 1998, Gen. Pharmac. 31:667-674; copy attached), which was prior to the priority date of the present application, and which provides these full names for the corresponding acronyms (see page 667, col. 1).

Withdrawal of these rejections is respectfully requested.

Art of Interest

The Office Action indicated that while no rejection under §102 or §103 had been made, that applicants were to note a number of references, and pointed to a packet entitled "Art of Interest".

Unfortunately, no such packet was included with the copy of the Office Action as received. There is also no list of references included in this packet that might facilitate applicants' review of such.

Applicants respectfully request that this packet of "Art of Interest" be forwarded with the next action, and that these references be made of record in the present application by listing them in the "Notice of References Cited" (form PTO-892).

Applicants respectfully request that, should the Office find the present claims encompass the prior art, that any such rejection be made so that applicants may have the opportunity to address such. Such a rejection should be non-final, as no prior art rejection has been made against the claims to date.

Conclusion

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number ASIL-002CIP.

Respectfully submitted,
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Date: Aug 7, 2003

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Enclosures: Shieh et al. (1997 Curr. Biol. 7:R627-630)
Leβmann et al. (Aug. 1998, Gen. Pharmac. 31:667-674)

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Neurotrophins: New roles for reasoned cast

Perry B. Shieh and Anirvan Ghosh

Recent studies suggest that endogenous neurotrophins play a central role in the patterning of cortical connections and in cortical synaptic physiology. Do these effects of neurotrophins reflect independent cellular events, or are they manifestations of a single cellular mechanism central to developmental plasticity?

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Much of our understanding of the activity-dependent development of the cortex has been derived from studies of thalamic projections to the visual cortex. Axons from the lateral geniculate nucleus (LGN) of the thalamus are initially intermixed within layer 4 of the visual cortex. A process of eye-specific axon segregation leads to the formation of ocular dominance columns, which include groups of neurons that respond preferentially to stimulation of one eye (Figure 1). Monocular deprivation during a restricted period in development — called the critical period — leads to a shift in ocular dominance columns in favor of the non-deprived eye [1]. The formation of ocular dominance columns can be prevented by intraocular injection of tetrodotoxin, a sodium channel blocker, suggesting that the process requires action potential activity [2]. It has also been shown that blockade of the *N*-methyl-D-aspartate (NMDA) class of glutamate receptors in the cortex prevents ocular dominance plasticity, indicating a role for synaptic transmission in this process [3]. There has been much interest over the past few years in identifying the molecular mechanisms that mediate these activity-dependent changes in the developing cortex.

One class of molecules that has been suggested to play a role in the development of thalamocortical projections is the neurotrophin family of growth factors. Neurotrophins are small, secreted proteins that have been found to play important roles in various aspects of nervous system development. Members of this family, which includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), were initially identified as factors that promote the survival of various classes of neurons. As discussed below, a number of recent studies have provided evidence that neurotrophins can also exert marked influences on cellular events that may be central to cortical plasticity.

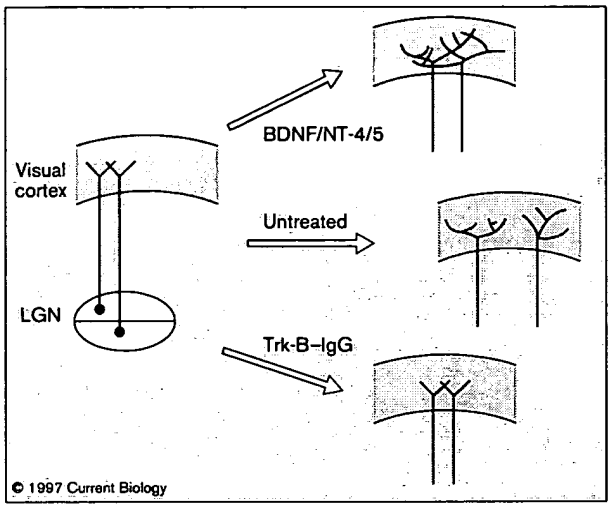
Neurotrophins and thalamocortical patterning

A long-standing hypothesis concerning the patterning of axonal projections holds that developing axons require target-derived factors for their survival, and that ingrowing axons compete for limiting amounts of such target-derived trophic factors. It has also been proposed that, during thalamocortical development, more active thalamic afferents have a competitive advantage. One class of molecules that may serve as target-derived trophic factors are the neurotrophins. A prediction of this possibility is that an excess of target-derived neurotrophins should eliminate competition among thalamic afferents and, indeed, Cabelli *et al.* [4] have shown that infusion of BDNF or NT-4/5 into the visual cortex of kittens during the critical period prevents the segregation of geniculocortical axons into ocular dominance columns.

Although this observation indicates that exogenously applied neurotrophins can influence the patterning of thalamic axons in the cortex, it does not show whether endogenous neurotrophins have a similar function. Cabelli *et al.* [5] have recently addressed this question by infusing neurotrophin 'receptor-bodies' into the visual cortex during the critical period. Receptor-bodies are fusion proteins consisting of the ligand-binding domain of a receptor — in this case one of the neurotrophin receptors, Trk-A, Trk-B or Trk-C — fused to the Fc portion of human immunoglobulin G (IgG). They found that infusion of Trk-B–IgG, but not Trk-A–IgG or Trk-C–IgG, into the visual cortex of kittens blocked the segregation of LGN axons into ocular dominance columns. This observation suggests that a Trk-B ligand, most likely BDNF or NT-4/5, participates in the eye-specific segregation of geniculocortical axons (Figure 1).

These elegant experiments suggest that endogenous neurotrophins play an important role in the patterning of thalamocortical projections, but the specific cellular function of the neurotrophins in this process is not easily inferred. One possibility is that BDNF or NT-4/5 acts principally to regulate the growth of thalamic axons within layer 4. Neurotrophin infusion may lead to an increased growth of thalamic axons within layer 4, and Trk-B–IgG infusions may arrest the ingrowing axons in an immature, unsegregated state. Alternatively, BDNF or NT-4/5 may play a central role in activity-dependent synaptic plasticity and may mediate the stabilization or growth of appropriate synaptic contacts. Infusion of neurotrophins may lead to the stabilization of inappropriate synapses, and the sequestration of neurotrophins by Trk-B–IgG receptor-bodies might prevent all forms of activity-dependent

Figure 1



Diagrammatic representation of the effects of neurotrophin perturbation on ocular dominance column formation. Infusion of BDNF, NT-4/5 or Trk-B–IgG prevents the formation of ocular dominance columns, but the effects of these perturbations on individual LGN axons may be distinct, as shown here. See text for details.

rearrangements from taking place. Both of these potential mechanisms are consistent with the results of the neurotrophin perturbation experiments. Moreover, as discussed below, emerging evidence suggests that neurotrophins can also have major effects on the dendritic development of postsynaptic cortical neurons, which could indirectly influence the elaboration of presynaptic axon terminals.

Neurotrophins and dendritic growth

In a recent series of experiments, McAllister and colleagues [6,7] have made some interesting observations regarding the influence of neurotrophins on dendritic development in cortical slice cultures. In their initial study [6], the authors reported that cells in different layers of the cortex respond differentially to neurotrophin stimulation. For example, BDNF promotes the growth of dendrites of layer 4 and 5 neurons, but suppresses the development of basal dendrites in layer 6. They also noted that cells in a given layer respond distinctly to stimulation by different neurotrophins. Layer 4 neurons, which respond positively to BDNF stimulation, are virtually unaffected by NT-3 stimulation. These findings indicate that neurotrophins can have marked layer-specific effects on the dendritic development of cortical neurons.

These observations have recently been extended to include an analysis of the role of endogenous neurotrophins in the regulation of dendritic growth. By using receptor-bodies to inhibit the function of endogenous neurotrophins, McAllister *et al.* [7] found that treatment of cortical slices with Trk-B–IgG or Trk-C–IgG had opposite effects on the same population of neurons. Whereas Trk-B–IgG treatment inhibited dendritic growth of layer 4 neurons, Trk-C–IgG treatment promoted it, suggesting that the endogenous Trk-B ligands, BDNF and NT-4/5, and the Trk-C ligand, NT-3, have opposing effects on the dendritic growth of layer 4 neurons.

This result is particularly striking, as stimulation of both Trk-B and Trk-C receptors leads to the activation of the Ras–mitogen activated protein (MAP) kinase signal transduction cascade, and therefore would be expected to have similar cellular consequences. The distinct effects of inhibiting BDNF and NT-3 strongly suggest that there are important differences in the Trk-B and Trk-C signaling pathways that remain to be identified. Although it is not yet clear whether neurotrophins can influence dendritic growth during the critical period, given the findings of the slice culture experiments that possibility must be taken into account in interpreting the results of neurotrophin or Trk-B–IgG infusion experiments described above.

Neurotrophins and synaptic plasticity

The cellular effects of neurotrophins on thalamic axons and cortical dendrites are seen over the course of days, and therefore do not reveal whether these factors can have acute effects on the physiology or function of cortical neurons. In the past few years, this possibility has received much attention from experiments that have examined the role of neurotrophins in synaptic plasticity. In 1995, Kang and Schuman [8] reported that acute treatment of adult hippocampal slices with BDNF or NT-3 led to a rapid enhancement of synaptic transmission, and that this form of synaptic enhancement did not occlude long-term potentiation (LTP), a form of synaptic plasticity that has been extensively investigated in the hippocampus and neocortex as a cellular model of learning and memory.

This observation generated a great deal of excitement, as well as some level of controversy, in part because some subsequent reports were not entirely consistent with this finding. For example, Figurov *et al.* [9] reported that BDNF promoted the induction of LTP in young, but not adult, hippocampal slices. Moreover, they found that, in the absence of activity, BDNF did not affect the efficacy of basal synaptic transmission. Although these differences have generally been attributed to variations in the details of the experimental procedure, they underscore the point that there is not yet universal agreement on the specific role of neurotrophins in the modulation of synaptic transmission.

A second line of evidence that supports a role for BDNF in synaptic plasticity has come from analysis of mice with a targeted disruption of the BDNF gene. Two different groups have reported that LTP is impaired in BDNF null mice [10,11]. Importantly, this defect can be rescued by

BDNF provided either by retroviral infection of hippocampal slices [12] or by bath application of BDNF [11], suggesting that the defect is likely to be related to an acute requirement for BDNF rather than being a developmental consequence of the absence of BDNF. It is also noteworthy that the heterozygous animals also show defective LTP, suggesting that the levels of BDNF may be an important determinant of synaptic plasticity.

Recent observations suggest that, as in the hippocampus, plasticity in the cortex can also be modulated by neurotrophins. Two findings in this regard are particularly noteworthy. Akaneya *et al.* [13] have reported that application of recombinant BDNF can prevent the induction of long-term depression (LTD) in layer 2/3 of cortex in response to low frequency stimulation of layer 4. More recently, the same group [14] found that treatment with BDNF — but not NT-3 or NGF — could lead to an enhancement of field potentials recorded in layer 2/3 following layer 4 stimulation. They also found that bath application of Trk-B–IgG or K252a (a Trk receptor family inhibitor) could prevent the induction of LTP in these slices, suggesting that endogenous BDNF may be required for certain forms of synaptic plasticity in cortical slices. Although these findings require further investigation, they suggest that neurotrophins can have rapid and pronounced effects on cortical synaptic physiology.

Perspectives

The recent evidence that neurotrophins modulate both synaptic plasticity and cortical development has renewed interest in the possibility that synaptic plasticity and activity-dependent ocular dominance plasticity may share common underlying mechanisms. One interpretation of these findings is that neurotrophins directly contribute to synaptic plasticity, and that these synaptic changes in turn are responsible for the activity-dependent remodeling of thalamocortical axons that is the basis of ocular dominance column segregation.

This model is appealing, because it unifies the mechanism underlying two forms of activity-dependent plasticity. Recent studies in the rat somatosensory [15] and visual [16] cortex, which show that LTP can be induced during a time that coincides with the critical period and that the two events have similar pharmacological responses, provide indirect support for this possibility. It should be noted, however, that such correlations do not prove that synaptic plasticity and activity-dependent reorganization of thalamic axons are causally related events, and further work is necessary before such a conclusion can be drawn.

With regard to the effects of neurotrophins on the activity-dependent reorganization of thalamic afferents, there are several important issues that remain to be addressed. It will be of particular interest to explore how BDNF or NT-4/5 might contribute to the growth or stabilization of appropriate thalamic axons. One possibility is that, in the postsynaptic neuron, the neurotrophin is transported to active synapses, leading to specific stabilization of those synapses. Another possibility is that BDNF or NT-4/5 is released by the postsynaptic neuron in a relatively non-specific way and acts as a permissive signal for activity-dependent synaptic plasticity. A third possibility is that active thalamic axons are more effective in responding to target-derived neurotrophins, even if the factors are not released in a synapse-specific manner. These possible modes of action by which neurotrophins might affect synaptic physiology and afferent growth are not mutually exclusive, and additional experiments are required to determine whether one or more of these mechanisms are indeed involved.

Another issue that needs to be addressed is the electrophysiological response of cortical neurons *in vivo* under conditions of neurotrophin or Trk-B–IgG infusion. Given the extensive evidence that inhibition of neuronal activity can prevent ocular segregation, it is important to determine whether perturbation of neurotrophins has acute effects on synaptic physiology *in vivo*. It would also be interesting to know whether the effects of monocular deprivation on the patterning of thalamic afferents can be rescued by providing exogenous neurotrophins. Monocular deprivation typically leads to the retraction of thalamic axons receiving inputs from the deprived eye. If this is because of their inability to access target-derived BDNF or NT-4/5, then infusion of these neurotrophins should rescue this defect. A failure to rescue would be more consistent with the possibility that BDNF acts as a permissive factor for ocular dominance plasticity. The answers to these questions will be critical as we try to understand the specific function of neurotrophins in cortical plasticity.

The observations from recent neurotrophin perturbation experiments have provided important evidence in support of a role for these factors in cortical development. Despite this progress, we are still far from understanding the mechanisms by which neurotrophins affect various cellular events in the cortex, and from knowing whether they are manifestations of a single neurotrophin-regulated cellular event. It will be of interest to see which of the many current hypotheses survive more rigorous investigations, which are sure to come in short order.

Acknowledgement

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REVIEW

Neurotrophin-Dependent Modulation of Glutamatergic Synaptic Transmission in the Mammalian CNS

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ABSTRACT. 1. The protein family of the neurotrophins, consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and Neurotrophin-3, -4/5, and -6 (NT-3; NT-4/5; NT-6) is well known to enhance the survival and to stabilize the phenotype of different populations of neurons in the central and the peripheral nervous system. These effects are mediated via binding to specific tyrosine kinase receptors (Trks) and to the low-affinity p75 neurotrophin receptor.

2. The neurotrophins NGF, BDNF, and NT-3 and the BDNF and NT-3 selective receptors (TrkB, TrkC) are expressed at high levels in neurons of the basal forebrain, the hippocampus, and the neocortex of the mammalian brain. The expression and secretion of NGF and BDNF in these brain areas is regulated by (physiological levels of) neuronal activity.

3. Exogenous application of the neurotrophins to hippocampal and neocortical neurons can enhance excitatory glutamatergic synaptic transmission via activation of Trk receptors. In addition, long-term potentiation (a potential cellular correlate for learning and memory formation in mammals) in the rodent hippocampus depends on endogenous supply of neurons with BDNF.

4. Judged by the analysis of electrophysiological data, the BDNF- and NT-3-induced enhancement of glutamatergic synapses is mediated by increasing the efficacy of glutamate release from the presynaptic neuron. However, neurotrophin-dependent postsynaptic enhancement of NMDA (but not AMPA) receptor responsiveness has also been shown.

5. On the molecular level, neither the pre- nor the postsynaptic modulation of glutamatergic synapses by neurotrophins is well understood. However, neurotrophins were shown to acutely affect intraneuronal Ca^{2+} levels and to influence molecular components of the transmitter release machinery, which could underly the presynaptic modifications, whereas BDNF-induced phosphorylation of NMDA-type glutamate receptors could account for the postsynaptic effects.

6. Taken together, these results suggest that the activity-dependent release of neurotrophins at frequently used synapses could modulate the synaptic efficacy at these junctions. Thus, neurotrophins might operate as locally released feedback modulators of synaptic transmission, and this could be a cellular correlate for certain aspects of information processing in the mammalian brain. GEN PHARMAC 31;5:667–674, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. Neurotrophins, BDNF, synaptic plasticity, long-term potentiation, Trk receptors, presynaptic enhancement, glutamate, Xenopus neuromuscular junction, ocular dominance

INTRODUCTION

The protein family of the neurotrophins comprises nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3, -4/5, and -6 (NT-3, NT-4/5, and NT-6, respectively). The neurotrophins are homodimeric proteins, which are secreted by neuronal target tissue (or neurons) and can promote neuronal survival and neurite outgrowth of neurons of the peripheral as well as of the central nervous system (CNS). In addition, neurotrophins can stabilize specific phenotypes of CNS neurons via regulation of gene expression. Furthermore, neurotrophins can activate different types of protein kinases and intracellular second messengers, thus also acutely modulating cellular functions (for an overview see Lewin and Barde, 1996).

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Two different types of neurotrophin transmembrane receptors, mediating neurotrophin signaling have been described: the family of neurotrophin tyrosine kinase (Trk) receptors (TrkA, TrkB, TrkC), and the low affinity p75 neurotrophin receptor ($p75^{LNTR}$). The different neurotrophins specifically bind to their cognate Trk receptors (TrkA, specific binding of NGF; TrkB, specific for BDNF and NT-4/5; and TrkC, specific for NT-3; reviewed by Barbacid, 1994). In addition, the different neurotrophins can bind with similar affinity to the nonselective $p75^{LNTR}$, which is shared by all neurotrophins. Most of the biological effects of the neurotrophins are mediated either by Trk receptor signaling alone or by the conjoint activation of Trk and $p75^{LNTR}$ receptors (Barbacid, 1994; Chao, 1994), leading to the activation of a complex network of intracellular signaling cascades, including induction of transcription factors (e.g., c-fos) and the activation of different classes of serine/threonine [e.g., mitogen activated protein (MAP) kinases] as well as of intracellular tyrosine

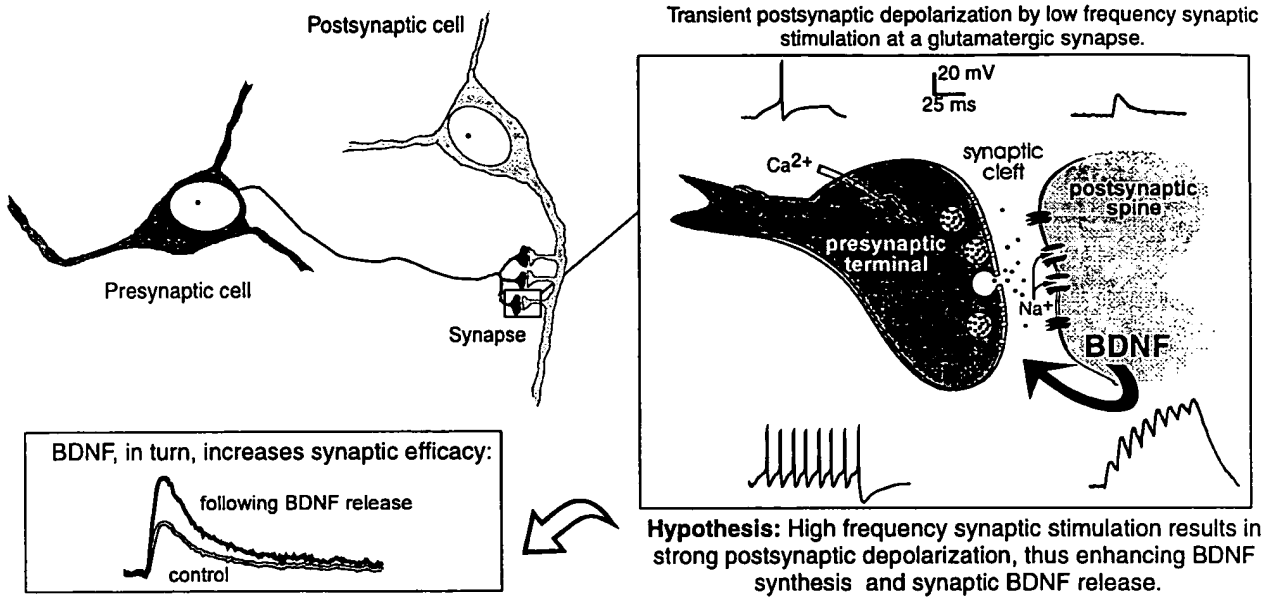

FIGURE 1. The diagram depicts the hypothesized role of BDNF for activity-dependent enhancement of glutamatergic synapses in the mammalian CNS. CNS neurons communicate via chemical synapses (upper left). At glutamatergic synaptic terminals (right), high-frequency synaptic stimulation leads to depolarization-induced increased BDNF synthesis and secretion. The released neurotrophin enhances the efficacy of the activated synapse via pre- or postsynaptic mechanisms, thus increasing the postsynaptic response to single synaptic stimuli (lower left).

kinases (reviewed by Heumann, 1994). In addition, the $p75^{LNTR}$ can activate separate intracellular signaling cascades independent from Trk receptor signaling (Dechant and Barde, 1997).

Besides the long-range effects of neurotrophins leading to the support of neuronal survival and function (reviewed by Thoenen, 1991), evidence has accumulated in recent years that neurotrophins might serve as feedback regulators for the efficacy of synaptic transmission in the mammalian CNS (see Fig. 1). It was shown that neurotrophins are synthesized in an activity-dependent manner by CNS neurons (Zafra *et al.*, 1992; Patterson *et al.*, 1992; Castren *et al.*, 1993; Dragunow *et al.*, 1993) and that neurotrophins are released upon depolarization of CNS neurons (Blöchl and Thoenen, 1995, 1996; Goodman, 1996). Thus, neurotrophins are synthesized and released in an activity-dependent manner, making them ideally suited to influence the electrical neuronal activity regulating their own synthesis/release. The pioneering report by Lohof *et al.* (1993), showing the neurotrophin-dependent enhancement of cholinergic synaptic transmission at the Xenopus neuromuscular junction prompted a number of studies trying to extend these findings to excitatory synaptic transmission in the mammalian CNS. Indeed, recent results seem to directly support a contribution of neurotrophin-dependent processes to certain aspects of synaptic plasticity in the mammalian CNS.

Whereas several recent reviews provided important information with respect to the acute and long-term effects of neurotrophins on neuronal physiology in the mammalian CNS (Thoenen, 1995; Lewin and Barde, 1996; Bonhoeffer, 1996; Berninger and Poo, 1996; Cellerino and Maffei, 1996; Knipper and Rylett, 1997), this review will focus on the available (especially electrophysiological) data with respect to the modulation of glutamatergic synaptic transmission in the rodent CNS and will discuss the possible synaptic targets for the observed modifications.

Expression of neurotrophins and their receptors in the hippocampus and neocortex

In the rodent CNS, BDNF, NT-3, and NGF show prominent expresssion in the hippocampus and the neocortex (Ernfors *et al.*, 1992). Among the tyrosine kinase receptors for neurotrophins (NTs), TrkB (specific for BDNF and NT-4/5) and TrkC (specific for NT-3) are abundantly expressed in neurons of the hippocampus and the neocortex, whereas the NGF-specific TrkA receptor is virtually absent from neuronal somata resident in these brain areas. However, TrkA receptors are present on afferent cholinergic fibres from neurons of the basal forebrain projecting to the hippocampus and the neocortex, respectively (reviewed e.g., by Barbacid, 1994). Likewise, the $p75^{LNTR}$ neurotrophin receptor is also present on these afferent cholinergic fibres, whereas it is absent from neurons residing within the hippocampus and neocortex.

After identification of this set of neurotrophins and neurotrophin receptors, it rapidly emerged that, unlike their functions in the PNS, the NTs do not seem to have irreplaceable implications for neuronal survival in the CNS, as shown most elegantly in the neurotrophin and Trk receptor knockout-mice (reviewed by Snider, 1994). However, the activity dependent (i.e., depolarization-induced) regulation of neurotrophin expression in cortical neurons (reviewed by Lindholm *et al.*, 1994) and the depolarization-induced sequestration of NTs from hippocampal neurons (Blöchl and Thoenen, 1995, 1996; Goodman *et al.*, 1996) pointed to a critical role for NTs in response to intense electrical stimulation. In consequence, NTs are believed to have important implications for neuronal protection following several types of brain insults that are accompanied by detremental excitatory synaptic stimulation (reviewed in Lindvall *et al.*, 1994).

These findings were extended by several reports, showing that cortical BDNF expression is enhanced even in response to physio-

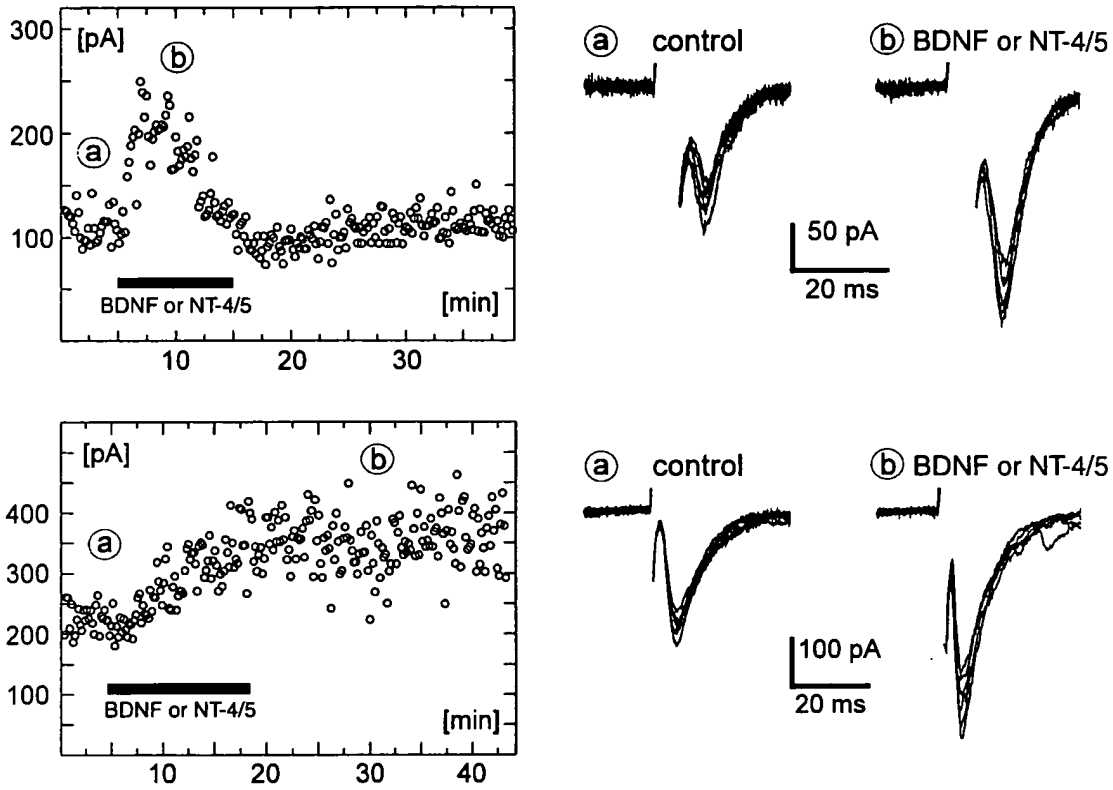

FIGURE 2. Exogenously applied neurotrophins enhance glutamatergic synaptic transmission. Exogenous application of either of the two TrkB receptor ligands (BDNF; NT-4/5) can enhance glutamatergic synaptic transmission in isolated cultured hippocampal pyramidal cells, leading either to transient (upper panel) or sustained (lower panel) increases in synaptic efficacy in different cells. Recordings of single cell-activated glutamatergic epscs from isolated hippocampal pyramidal cells in microculture (for experimental details see Leßmann and Heumann, 1998). Duration of application of neurotrophins is indicated by black bars. On the right, consecutive original current traces recorded at the time points indicated by "a" and "b" are shown. Similar changes in synaptic efficacy of glutamatergic epscs have been shown in hippocampal and neocortical slice preparations (see e.g., Kang and Schuman, 1995a; Carmignoto *et al.*, 1997; Akaneya *et al.*, 1997; Tanaka *et al.*, 1997).

logical levels of neuronal activity, such as tetanic stimulation of excitatory pathways inducing long-term potentiation (Patterson *et al.*, 1992, Dragunow *et al.*, 1993, Castren *et al.*, 1993) and sensory stimulation of cortical afferents (Castren *et al.*, 1992; Rocamora *et al.*, 1996). Thus, physiological levels of excitatory synaptic activity increase the expression of BDNF, the most abundant neurotrophin in the postnatal rodent hippocampus and neocortex. It remains to be shown whether the enhanced intracellular level of BDNF in response to excitation is accompanied by an increased release of BDNF into the extracellular space following synaptic stimulation. If this were to be true, feedback modulation of excitatory synaptic transmission by BDNF via the prominently expressed TrkB tyrosine kinase receptor in these brain regions could occur.

Modulation of glutamatergic synaptic transmission by exogenous BDNF, NT-4/5 or NT-3

The hypothesized role of NTs as feedback modulators of excitatory synaptic transmission in the hippocampus and neocortex prompted a number of studies investigating the acute effects of the neurotrophins on glutamatergic synapses (see Fig. 2). Starting in 1994, several groups have described an acute (i.e., onset within several minutes) BDNF-induced enhancement of glutamatergic synaptic transmission in low-density postnatal rat hippocampal cultures (Leßmann *et al.*, 1994; Leßmann and Heumann, 1998), in embryonic rat hippocampal mass cultures (Levine *et al.*, 1995a, 1996), in different excitatory pathways of adult rat hippocampal slices (CA1 region: Kang and Schuman, 1995a, 1996; Scharfman, 1997; CA3 region and dentate gyrus: Scharfman, 1997) and in slices of juvenile rat visual cortex (Carmignoto *et al.*, 1997; Akaneya *et al.*, 1997). In all cases, this modulation was shown to depend on the activation of the BDNF- (and NT-4/5-) specific TrkB tyrosine kinase receptor, whereas activation of the unselective low-affinity $p75^{LNTR}$ receptor was not involved. Interestingly, it was shown recently that BDNF also enhances glutamatergic synaptic transmission in the dentate gyrus of the hippocampus *in vivo* (Messaoudi *et al.*, 1998), emphasizing the physiological significance of synaptic BDNF signaling in the mammalian CNS.

The studies by Kang and Schuman (1995a, 1996) revealed an additional enhancement of glutamatergic synaptic transmission by NT-3, comparable to the effects induced by BDNF. However, in different hippocampal and in neocortical preparations, NT-3 failed to have similar effects on glutamatergic synaptic transmisson when compared to BDNF (Levine *et al.*, 1996; Figurov *et al.*, 1996; Akaneya *et al.*, 1997; but see Kim *et al.*, 1994), and it is not entirely clear whether the NT-3-induced enhancement observed by Kang and coworkers (1995a, 1996) could have been mediated by activation of TrkB receptors, known to be activated by high concentrations of

NT-3 (Barbacid *et al.*, 1994). However, the NT-3 selective TrkC tyrosine kinase receptors are definitely expressed in the rodent hippocampus and neocortex (Barbacid *et al.*, 1994), making NT-3-induced synaptic enhancement via activation of TrkC receptors equally likely. Clearly, additional data are necessary to address this issue.

In summary, these results show that BDNF, NT-4/5, and NT-3 can acutely enhance glutamatergic synaptic transmission in diverse hippocampal and neocortical preperations via activation of Trk receptors.

The electrophysiological studies discussed above also allowed to distinguish between pre- and postsynaptic modifications by neurotrophins in these preperations: Judged by the analysis of paired-pulse facilitation, of the amplitude and the frequency of miniature synaptic currents (mepscs), of the coefficient of variation of evoked excitatory postsynaptic current (epsc) amplitudes, and of synaptic failure rates (i.e., a measure for the reliability of action potentials to induce fusion of transmitter vesicles), the observed BDNF-induced enhancement is presynaptic in origin (Leßmann *et al.*, 1994; Kang and Schuman, 1995a; Carmignoto *et al.*, 1997; Leßmann and Heumann, 1998). Additional postsynaptic modifications are suggested to operate in parallel because intracellular application of the Trk tyrosine kinase specific inhibitor k252a into postsynaptic neurons of cortical network cultures inhibits the BDNF-induced synaptic enhancement (Levine *et al.*, 1995a). However, this could be due to inhibition of BDNF effects at additional synapses of the network, where the recorded "postsynaptic" neuron is the presynaptic partner. Thus, an originally presynaptic modulation could be converted into changes of the excitatory drive in the network, leading to altered spontaneous synaptic activity in the recorded neuron. In addition, recent work from this laboratory revealed that this postsnyaptic modification is confined to NMDA receptor mediated synaptic currents whereas the AMPA receptor mediated component remains unaffected (Levine *et al.*, 1998).

Taken together, several lines of evidence suggest a BDNF-induced presynaptic enhancement of glutamate release, although additional postsynaptic modifications of NMDA receptors can operate in parallel.

Although the BDNF-induced enhancement of glutamatergic synaptic transmission in the hippocampus has been shown unequivocally (see above), these data require to be compared carefully: The enhancement of glutamatergic epscs in the CA1 region of adult rat hippocampal slices, as described above (Kang and Schuman, 1995a; 1996), was not observed in similar studies of other groups under different recording conditions (i.e., slower penetration of BDNF into slices, use of younger animals, different recording site of extracellular epsps: Figurov *et al.*, 1996; Patterson *et al.*, 1996; Tanaka *et al.*, 1997). Indeed, Kang *et al.* (1996) have shown that especially the perfusion rate of BDNF in the CA1 experiments is a critical determinant to observe the acute enhancement of epsps in slices of this brain region. However, those groups that failed to see an acute modulation of epscs/epsps in the CA1 region still observed effects of BDNF in their preparation upon prolonged application of the neurotrophin: Figurov *et al.* (1996) reported that long-lasting (i.e., for several hours) incubation of juvenile hippocampal slices with BDNF can ameliorate the synaptic fatigue during repetitive stimulation, which is responsible for the lack of long-term potentiation (LTP) in slices from early postnatal animals. Patterson *et al.* (1996) showed that BDNF (incubation time >30 min.) can rescue the lack of LTP in the CA1 region of BDNF knockout mice (see below), whereas BDNF had no effect on basal synaptic transmission or generation of LTP in wild-type littermates. This selective lack of an effect of exogenously applied BDNF suggests that endogenously released BDNF in the wild-type mice could have obscured additional enhancement of exogenously applied BDNF. Thus, to observe an acute effect of BDNF on glutamatergic epscs it could be important to minimize release of endogenous BDNF before recording.

With respect to a possible role of neurotrophins for long-lasting activity-dependent changes in glutamatergic synaptic transmission (i.e., LTP; see below), it is important to consider the duration of neurotrophin-induced synaptic enhancement. In cultured preparations, the effects of neurotrophins were reported to be transient despite continued neurotrophin application (see e.g., Kim *et al.*, 1994; Leßmann *et al.*, 1994) or to be reversible upon washout of the respective neurotrophin (Levine *et al.*, 1995a). However, a recent study suggests that also enduring BDNF-induced enhancement of glutamatergic epscs, which is sustained upon washout of the neurotrophin, can be observed in cultured neurons (Leßmann and Heumann, 1998). The effects of BDNF on glutamatergic synaptic transmission in slice preparations have been shown to be long-lasting ($\geq$1 hr) and to be retained upon washout of neurotrophins (see e.g., Kang and Schuman, 1995a; Carmignoto *et al.*, 1997; Akaneya *et al.*, 1997). Interestingly, Tanaka *et al.* (1997) have also reported a transient enhancement of glutamatergic epsc amplitudes upon application of BDNF in the CA1 region of hippocampal slices. Taken together these results suggest that transient and sustained enhancement of glutamatergic epscs by BDNF can be observed in cultured neurons and in acute slice preparations. Whereas the sustained enhancement could correlate with long-lasting activity dependent changes during LTP, the significance of the transient effects and the effects that are reversible upon washout of neurotrophins for LTP phenomena is unclear.

In principle, it could be possible that the sustained enhancement in slice preparations results from ineffective washout of the very sticky neurotrophins. However, Akaneya *et al.* (1997) have directly shown succesful washout of neurotrophins within 5 min from neocortical slices. Thus, at least in the neocortical slice, the sustained enhancement of glutamatergic epscs by BDNF does not seem to result from residual levels of the neurotrophin within the tissue.

Although it is clear from the wealth of these data that BDNF has the potential to modulate the efficacy of glutamtergic synaptic transmission, the cellular mechanisms of the neurotrophin mediated synaptic modulation in these different preparations are less clear. This is partially due to the fact that in most of these electrophysiological studies the neurotrophins were applied to a complex neuronal network, and recordings were mostly obtained from neuronal populations rather than from single neurons. Thus, under these conditions, neurotrophin-induced changes of neuronal properties not directly related to glutamatergic synaptic transmission can induce secondary effects on the overall excitatory synaptic efficacy of the synaptic network. For example, it was shown that NT-3 inhibits gabaergic synaptic transmission in cortical cultures, leading in turn to increased glutamatergic network activity [Kim *et al.*, 1994; a similar decrease in gabaergic synaptic transmission by BDNF in hippocampal slices was shown recently to result from postsynaptic modifications of Gaba A receptor gating (Tanaka *et al.*, 1997)].

However, in microcultures consisting of single (pairs of) hippocampal neurons grown in isolation (Leßmann and Heumann, 1997), it was clearly shown that both TrkB ligands (BDNF and NT-4/5) have direct presynaptic effects on the efficacy of glutamatergic synaptic transmission (Leßmann and Heumann, 1998), thus ruling out secondary effects of the neurotrophins on the excitatory network activity to explain the neurotrophin-dependent synaptic modulation.

This direct presynaptic enhancement of excitatory glutamatergic synaptic transmission in cultured single (pairs of) mammalian CNS neurons parallels with a similar Trk receptor-dependent potentia-

tion of cholinergic neuromuscular synaptic transmission in isolated frog nerve-muscle cultures. In this preparation, BDNF, NT-3, and NT-4/5 presynaptically enhance synaptic transmission by increasing the probability of acetylcholine release within several minutes of neurotrophin application (Lohof *et al.*, 1993; Stoop and Poo, 1995, 1996). Likewise, chronic application (i.e., 3 days) of either BDNF or NT-3 to Xenopus nerve-muscle preparations leads to accelerated presynaptic maturation of the transmitter release machinery (Wang *et al.*, 1995), suggesting additional long-lasting presynaptic effects of neurotrophins on cholinergic synaptic transmission. The physiological significance of this finding was stressed by experiments, showing that muscle-derived NT-3 is an endogenous modulator of presynaptic maturation in this preparation (Xie *et al.*, 1997; Liou and Fou, 1997). Importantly, to date, all effects of neurotrophins on neuromuscular synaptic transmission have been shown to result exclusively from alterations of presynaptic function, suggesting a similar mechanism of modulation of excitatory synapses in neuromuscular and glutamatergic CNS preparations.

Modulation of glutamatergic synaptic transmission by exogenous NGF

Several studies have shown that NGF also can acutely modulate glutamatergic synaptic transmission in the rodent hippocampus and neocortex: Knipper *et al.* (1994a, 1994b) reported enhancement of synaptosomal transmitter release by NGF (and BDNF) from subcellular fractions of hippocampal tissue and described NGF-mediated enhancement of glutamatergic synaptic transmission in the rat hippocampus. Furthermore, NGF (as well as BDNF) was shown to enhance glutamatergic synaptic transmission in slices of rat visual cortex (Carmignoto *et al.*, 1997). Finally, NGF has been reported to interfere with activity-dependent changes of glutamatergic synaptic transmission in the rat hippocampus (Tancredi *et al.*, 1993; Ruberti *et al.*, 1997), and in the rat visual cortex (reviewed e.g., in Cellerino and Maffei, 1996; Bonhoeffer, 1996).

On the other hand, NGF has been shown to be ineffective in several hippocampal and neocortical preparations to modulate directly glutamatergic synaptic transmission in slice preparations (see e.g., Kang and Schuman, 1995a; Akaneya *et al.*, 1997) and in cultured neurons (see e.g., Levine *et al.*, 1995a; Leßmann and Heumann, 1998), whereas BDNF, NT-4/5, and NT-3 were effective. In light of the fact that the receptors that could interact with NGF (i.e., TrkA, $p75^{LNTR}$) are absent (Holtzmann *et al.*, 1995), or at least expressed at very low levels (Cellerino, 1995), in the rodent hippocampus and neocortex (see above), it is unclear whether NGF can have direct effects on glutamatergic synaptic transmission in these brain areas. It seems likely that NGF rather affects the cholinergic neurons (expressing TrkA and $p75^{LNTR}$ receptors), which project from the basal forebrain to cortical structures, thus indirectly influencing glutamatergic synaptic physiology by altering the cholinergic synaptic input in these brain areas.

Molecular mechanisms of enhancement of glutamatergic synaptic transmission

The definite molecular targets for the modulation of glutamatergic synaptic transmission by neurotrophins remain to be elucidated. However, several groups have described neurotrophin-induced modulation of cellular functions that could possibly influence the efficacy of glutamatergic synaptic transmission: It has been shown, that application of BDNF or NT-3 to cultured hippocampal neurons leads within minutes to increased levels of intracellular Ca^{2+}, which seems to be released primarily from internal Ca^{2+} stores (Berninger *et al.*, 1993; Canossa *et al.*, 1997). Such an elevation of intracellular Ca^{2+} levels could in turn lead to enhanced transmitter release at synaptic junctions. This view is supported by studies directly showing enhanced release of acetylcholine (Knipper *et al.*, 1994a) and glutamate (Knipper *et al.*, 1994b; Takei *et al.*, 1997) from isolated cortical synaptosomes upon acute stimulation with NGF. However, whether the enhanced release of transmitter from synaptosomes results from increased intracellular Ca^{2+} levels in this preparation remains to be shown. Interestingly, the BDNF-induced presynaptic enhancement of neuromuscular synaptic transmission in Xenopus has been shown to be correlated with increased presynaptic Ca^{2+} levels and to depend on the influx of Ca^{2+} from the extracellular space (Stoop and Poo, 1996). Furthermore, preliminary data point to an involvement of L-type voltage-gated Ca^{2+} channels in the BDNF- and NT-3-induced enhancement of glutamatergic epscs in the hippocampus (Kang and Schuman, 1995b). However, a direct causal connection between Ca^{2+} influx through L-type Ca^{2+} channels and neurotrophin-induced synaptic potentiation in the mammalian CNS remains to be shown.

Alternatively, enhanced release of transmitter could result independent from intracellular Ca^{2+} levels by direct modifications of proteins involved in the transmitter release machinery. In support of such mechanisms, two recent studies indicate that NGF and BDNF induce (MAP kinase dependent) phosphorylation of synapsin I (a membrane associated protein of transmitter vesicles, guiding their transport to the plasma membrane) at serine/threonine residues in cortical neurons (Knipper *et al.*, 1994b; Jovanovic *et al.*, 1996). However, it is not clear, whether synapsin I phosphorylation has a direct impact on glutamate release in these neurons.

Increased transmitter release from CNS neurons, as observed by electrophysiological recordings (see above) and biochemical studies on synaptosomes, could also result from enhanced presynaptic influx of Ca^{2+} from the extracellular space upon depolarization of the axon terminal. This could be achieved either directly by enhancing the function of voltage-gated Ca^{2+} channels or indirectly by prolongation of the presynaptic depolarization (and thus also Ca^{2+} channel activity) upon alterations of Na^+ and K^+ conductances. In this respect, it was shown, that at least NGF acutely (within minutes) enhances currents through voltage-gated Ca^{2+} channels in mouse dorsal-root ganglion neurons (Shen and Crain, 1994) and in molluscan neurons (Wildering *et al.*, 1995). In addition, culturing of embryonic basal forebrain neurons (Levine *et al.*, 1995b) and of a neuroblastoma cell line (Lesser and Lo, 1995) in the presence of NGF; and culturing of PC12 cells (expressing the respective Trk receptors) in the presence of NGF, BDNF, and NT-3 (see e.g., Tang Sherwood *et al.*, 1997) has been shown to increase the amplitudes of voltage-gated Ca^{2+}, Na^+, and K^+ currents in these preparations. However, it is unlikely that such an enhancement of voltage-activated ion currents upon chronic neurotrophin application can account for the acute modulation of glutamatergic synaptic transmission in hippocampal and neocortical preparations.

Thus, although these studies reveal the potential of neurotrophins to modulate, in principle, neuronal excitability in different neuronal (model) systems, acute effects of BDNF on voltage-gated ion channels in neurons in which synaptic transmission is enhanced by this neurotrophin have not yet been described. To date, the only study describing effects of neurotrophins on voltage-gated ion channels in cortical neurons has shown that acute application of NT-3 increases currents through Ca^{2+} activated K^+ channels (Holm *et al.*, 1997). At the synaptic level, such an effect would lead to a decrease rather than to an increase in presynaptic excitability, and it can not explain enhanced glutamatergic synaptic transmission in cortical neurons.

In conclusion, a causal connection between specific presynaptic alterations and the modulation of glutamatergic synaptic transmission within the same preparation remains to be shown.

All the work described until here focussed on modifications that could account for presynaptic modifications of glutamatergic synaptic transmission as observed in several synaptic preparations (Leßmann *et al.*, 1994; Kang and Schuman, 1995a; Carmignoto *et al.*, 1997; Leßmann and Heumann, 1998). A postsynaptic modulation of glutamatergic synaptic transmission as described by Levine *et al.* (1995a) could be mediated by enhancing the efficacy of postsynaptic glutamate receptor function. In this respect, it has been shown that TrkB receptors are present in the postsynaptic density of isolated synaptosomes from cortical neurons (Wu *et al.*, 1996) and that application of BDNF to these synaptosomes increases NMDA receptor phosphorylation (Suen *et al.*, 1997). This finding is of particular interest because serine/threonine as well as tyrosine phosphorylation of NMDA receptors have been shown previously to enhance NMDA receptor function (see e.g., Wang *et al.*, 1994; Wang and Salter, 1994), and BDNF-mediated modulation of NMDA receptor gating has been described recently (Levine *et al.*, 1998). In addition, enhancement of NMDA receptor function by direct interaction of neurotrophins (including BDNF) with NMDA receptors has been shown to operate under suboptimal concentrations of the NMDA coagonist glycine (Jarvis *et al.*, 1997). Thus, BDNF-dependent enhancement of NMDA receptor function could account for the postsynaptic modulation of NMDA receptor-mediated synaptic transmission in network cultures (Levine *et al.*, 1995a, 1996), whereas AMPA receptors are not affected by a similar postsynaptic mechanism (Levine *et al.*, 1998). Therefore, postsynaptic modifications of NMDA receptors are not related to the presynaptic enhancement of AMPA receptor-mediated unitary (i.e., single-cell activated) synaptic transmission in single (pairs of) cultured neurons (Leßmann and Heumann, 1998).

Significance of neurotrophins for activity-dependent synaptic plasticity: LTP

Long-term potentiation is a long-lasting enhancement of glutamatergic synaptic transmission in the rodent hippocampus and the neocortex, following high-frequency synaptic stimulation of specific afferents (for a review, see Bliss and Collingridge, 1993). In the light of (1) the activity dependent increase in neuronal BDNF mRNA, (2) the depolarization-induced secretion of BDNF into the extracellular space, and (3) the potential of BDNF to enhance glutamatergic epscs, it is tempting to speculate, that BDNF could be released from neurons upon high-frequency synaptic stimulation, thus mediating LTP (see Fig. 1). Although activity-dependent synaptic secretion of BDNF remains to be shown, several studies suggest that the supply of hippocampal and neocortical neurons with BDNF is a prerequisite to elicit LTP: It was shown independently in two different strains of transgenic mice with a targeted deletion of the BDNF gene that LTP in the CA1 region of the hippocampus is impaired but can be restored within several hours of exogenous application of BDNF (Korte *et al.*, 1995, 1996; Patterson *et al.*, 1996). In addition, the generation of LTP by tetanic stimulation of glutamatergic synapses in the visual cortex is facilitated in the presence of added BDNF (Akaneya *et al.*, 1997). Furthermore, the onset of succesful induction of LTP in the juvenile hippocampal CA1 region is correlated with the endogenous expression of BDNF in this area, and the failure to induce LTP in young animals, which is due to the fatigue of presynaptic terminals to release sufficient amounts of glutamate upon high-frequency stimulation, can be overcome by exogenous supply with BDNF (Figurov *et al.*, 1996). Finally, Kang *et al.* (1997) have shown that, in the CA1 region of the hippocampus, nominally LTP-generating synaptic stimulations at submaximal frequencies are ineffective in slices that had been pretreated with anti-BDNF function-blocking antibodies, directly correlating the generation of LTP with secretion of endogenously produced BDNF from intensely stimulated cells into the extracellular space. However, tetanic stimulation of CA1 synapses at maximal frequencies still generated LTP in the presence of these BDNF-antibodies, which might indicate the inability of the antibodies to buffer extracellular BDNF concentrations exceeding a critical value.

The study by Kang *et al.* (1997), employing BDNF function-blocking antibodies, and the investigation of BDNF-knockout mice by Korte *et al.* (1995) have also shown severe impairment of a later phase of maintenance of LTP, starting $\geq$1 hr after tetanic stimulation. Thus, endogenously released BDNF might participate not only in the rapid enhancement of glutamatergic epscs immediately following the induction of LTP (i.e., early LTP lasting $\leq$1 hr, which is mediated by posttranslational modifications), but it could also participate in the generation of "late LTP" (see Bliss and Collingridge, 1993), which lasts for at least 3 hr and is dependent on *de novo* protein synthesis.

Thus, it is clear from a number of studies that induction of LTP in the hippocampus and neocortex is correlated with the extracellular supply of neurons with BDNF, and it seems likely that BDNF is released by intensely stimulated neurons to provide positive feedback at glutamatergic synapses (see Fig. 1).

As shown by the localization of a BDNF/GFP fusion protein in transfected cortical neurons, BDNF containing secretory granules of the regulated pathway of protein secretion are indeed accumulated in the vicinity of synaptic junctions, providing for the first time direct evidence that BDNF is localized appropriately to function as activity dependently released synaptic messenger (Haubensak *et al.*, 1998).

Modulation of functional cortical plasticity by neurotrophins

Whereas this review focusses on direct modulation of glutamatergic synaptic transmission by neurotrophins (especially BDNF), several studies have described modulation of integrative properties of neuronal ensembles *in vivo* by neurotrophins. For example, Cabelli *et al.* (1995, 1997) have shown that BDNF participates in the formation of ocular dominance columns in the cat visual cortex. The ocular dominance columns represent neuronal ensembles that are excited predominantly by visual stimuli (which are relayed in the thalamus) to one eye. During development, these columns are built up by activity-dependent competition between the thalamic afferents from both eyes. It is believed that the thalamic (glutamatergic) afferents of both eyes compete for limited quantities of a trophic factor at synaptic contacts, thus stabilizing only the synaptic contacts of the thalamic afferents of one of the two eyes (for a review, see Katz and Shatz, 1996). Cabelli *et al.* (1995) have shown that infusion of BDNF into the developing visual cortex of kittens inhibits the formation of ocular dominance columns, most likely by impairing the competition of the thalamic afferents for limited trophic support due to the excess BDNF application. Likewise, it was reported recently that infusion of BDNF function-blocking immunglobulins into the visual cortex (which trap endogenously released BDNF) can also impair ocular dominance column formation (Cabelli *et al.*, 1997), directly showing that release of endogenous BDNF is an important prerequisite for this type of activity-dependent functional plasticity.

It seems likely that BDNF-mediated synaptic plasticity at glutamatergic synapses is the underlying mechanism at least of this type of functional plasticity in the visual cortex.

In addition, it has been shown that acute application of BDNF (and NGF) can rapidly modulate the functional representation of stimulated whiskers in the rat barrel cortex (Prakash *et al.*, 1996) and that intraventricular infusion of neurotrophins can ameliorate age-dependent learning deficits in rats (Fischer *et al.*, 1994), also pointing to direct modulation of information processing in the brain by neurotrophins. However, the cellular mechanisms of these effects are far from being understood, and additional studies are needed to establish a causal connection between neurotrophin-dependent synaptic plasticity and learning and memory formation.

CONCLUSIONS

The data reviewed in this article strongly support the notion that neurotrophins (especially BDNF) are mediators of synaptic plasticity in the mammalian central nervous system: Neurotrophins are synthesized in and secreted from CNS neurons in an activity-dependent fashion and can enhance excitatory synaptic transmission, creating a feedback loop between synaptic activation and strengthening of the activated synapses. Although this concept of neurotrophins as endogenously employed modulators of synaptic efficacy becomes increasingly clear, the information regarding the molecular determinants of neurotrophin-dependent synaptic plasticity is very sparse. Thus, future studies will have to focus on the intracellular signaling cascades involved to relate neurotrophin-induced synaptic changes with different forms of synaptic plasticity employing seperate or partially overlapping intracellular signaling mechanisms.

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